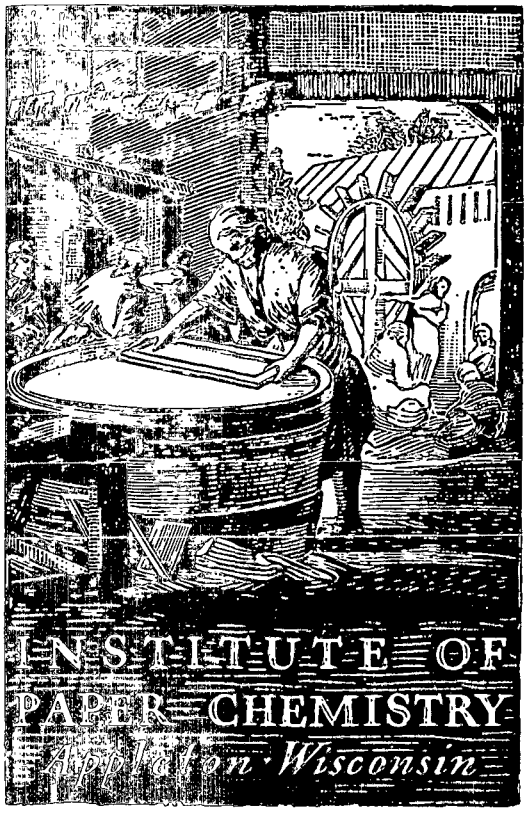

AN EVALUATION OF SUBLETHAL BIOASSAY METHODOLOGIES

PART 3. BLUEGILL RESPIRATION RATE AS A RAPID SUBLETHAL TOXICITY STRESS TEST FOR PAPER MILL WASTES

Project 3355

Report Four
A Progress Report
to
MEMBERS OF THE INSTITUTE OF PAPER CHEMISTRY

July 30, 1982

TABLE OF CONTENTS

THE INSTITUTE OF PAPER CHEMISTRY

Appleton, Wisconsin

AN EVALUATION OF SUBLETHAL BIOASSAY METHODOLOGIES

PART 3. BLUEGILL RESPIRATION RATE AS A RAPID SUBLETHAL TOXICITY STRESS TEST FOR PAPER MILL WASTES

SUMMARY

Changes in bluegill respiration rate in response to stress were investigated as a potential sublethal bioassay method for use with pulp and paper mill effluents. This method has the advantage of providing the rapid response lacking in conventional fish life cycle assays and would also allow assays of material available only in small volumes, such as experimental effluents.

The present work was done to establish base-line responses and tune the measuring apparatus. Five runs were made, all with diluted water only. Information was collected on the diurnal variability, the fish-to-fish variability, the effect of fish size on respiration rate, and the acclimation period.

Results to date indicate that fish have highly individual respiration rates. Similar fish in dilution water under similar conditions had significantly different rates. These rates did not vary significantly from day to day for individual fish. Differences in fish size, by weight, were not correlated with differences in respiration rates.

Bluegill showed different rates over a 24-hour period, with significant differences between day and night.

This method appears to have merit as a sublethal toxicity assay but probably would not be suitable for use as a routine monitoring device on site at a mill unless special facilities and personnel were provided.

INTRODUCTION

BACKGROUND

One of the three sublethal toxicity test methods selected for evaluation under Project 3355 was fish respiration. This test measures the presence of a sublethal toxicant by measuring changes in the rate of opercular movement of individual fish (usually bluegill sunfish) exposed to a waste stream. Irritation caused by a toxicant theoretically causes the fish to open and close its gill covers at a more rapid rate, probably in order to flush the gill epithelium or to improve oxygen uptake. This activity can be measured electronically, and significant increases in respiration rates can be interpreted as a response to the presence of a sublethal toxicant.

Interest in respiratory responses as a stress indicator began to draw attention in the 1960's, especially in circumstances where low dissolved oxygen was a factor (1). One of the first applications of respiration rate changes to a pollutant was made by Schaumburg et al. to measure DDT and untreated pulp mill effluent using young salmon (2). Several other studies were also done which demonstrated that pulp mill effluent affected ventilation rates. Water flow and quantitative tests were developed using the "cough response" as measured by a buccal catheter (3,4). For salmon the threshold concentration of kraft pulp mill effluent that elicited a cough response was about 20% of the 96-hour static LC50 (4). Tests were performed only on untreated effluents which were also acutely toxic.

The use of fish respiration or breathing rates became a more practical tool with the advent of a method which used a polygraph to record a signal obtained from an implanted electrode (5,6). Others discovered that a chamber containing external electrodes worked just as well at detecting a breathing signal and avoided the

necessity of implanted electrodes (7,8,9,10). These electrodes apparently detect small changes in electrical potential, which can be amplified and measured. The source of this change has been attributed both to changes caused by movements in water (7) and respiratory muscle potentials (8) and that a choice between a wire electrode or a mesh electrode will determine whether water movement will affect the signal. The mesh electrode will not record water moved by a stick placed in the chamber (8).

More recently this technique has been automated and computerized, which greatly facilitates the collection of the large number of data points needed to establish the normal daily fluctuations in breathing rates. Formerly, these observations were made visually or on a polygraph or recorder that required manual operation (11-4). One predecessor to the computerized system even used motion picture cinematography to collect the data (15). The computerized system has been applied to at least one industrial site where it is used as an early warning system whereby changes in ventilatory rates alert plant operators when effluents are out of specification (16).

The most immediate disadvantage of this high technology system is the cost. The particular example cited would require an estimated $75,000 to duplicate (16). Also, the reliability, cost effectiveness, and ecological significance of these systems have yet to be determined.

PROJECT OBJECTIVES

Because fish respiration rate data can still be collected with less sophisticated equipment, respiration rate was included as a method for evaluation under Project 3355. Data collected by this method were to be compared with data collected by other methods of sublethal stress analysis for information on the method's

precision as well as ecological significance. Little information exists in the literature which documents the significance of changes in respiratory rates on long-term health or production of fish or on ecosystem responses.

The immediate objectives of this work were to determine (a) whether the method could be employed in our laboratory to measure stress, (b) to identify factors that influenced the results, (c) and to optimize a procedure for collecting and analyzing bluegill respiratory rate data.

The following report details the progress made to date. This program has been under way for only a short time; the data produced are preliminary and were generated on dilution water only. The data are included with this report to provide information to any reader who may be contemplating further work with this assay and who may benefit from the effort completed to date.

MATERIALS AND METHODS

The respirometer used was assembled by a student, Jeff Brown, as part of his student research activities at the Institute. The apparatus includes six acrylic monitoring tanks joined in parallel enclosed by a wood framework, and covered with an acrylic lid (Fig. 1). Inserts were made that reduced the enclosed space and allowed the use of smaller or larger fish, depending on availability. A preaeration chamber allowed the influent solution to be aerated before it entered the fish chamber.

The test solution was provided on a flow-through basis through a baffled inlet and an overflow standpipe. Solutions were transferred from a storage container by "Masterflex" (Cole Parmer Co.) peristaltic pumps at a rate of 7-10 mL/min. Aeration of the chamber was accompished by using compressed air and a capillary pipette for the introduction of small bubbles. This procedure is optional; the solution may be aerated prior to delivery to the fish.

Electrodes were made of stainless steel mesh which completely spanned the width of the chamber. The electrode signal was amplified by a preamplifier as described by Drummond and Dawson (17). A rotary switch was used with the preamplifier to select input from any of the six chambers. The preamplifier also included two signal control adjustments.

The amplified signal was fed to an "Omniscribe" strip chart recorder which was set at 0.1-mV range and allowed variable operating speeds.

The apparatus was set up in a climate-controlled room at constant temperature (21°C), with a controlled light-dark photoperiod (as determined by the experiment), and enclosed in an opaque chamber to prevent visual stimuli and operator movements from alarming the fish. The chambers were on an elevated surface and a

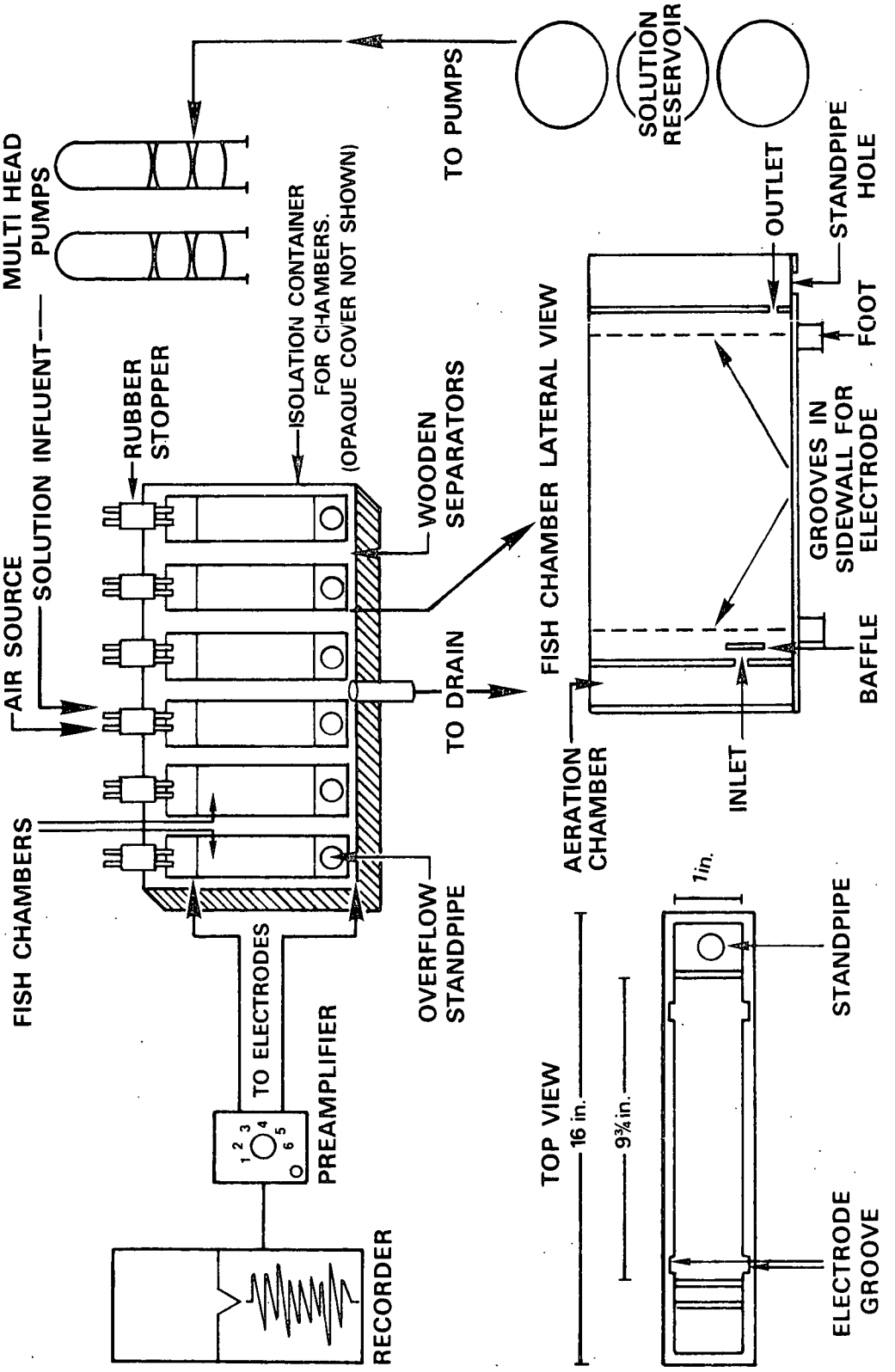

Figure 1. Fish respiration monitoring apparatus.

foam pad to reduce vibrations. However extraneous activities such as slamming doors and using electric drills caused occasional artifacts in the respiration rate data.

Fish were acclimated to the test conditions by transferring them to a small aquarium in the controlled climate room 48 hours prior to a test. The fish were then transferred to the chambers, and electrodes and covers were added. The covers were left undisturbed for the duration of the experiment.

Respiration rates were recorded over an interval of 3-5 minutes for each fish at the frequency dictated by the experiment (hourly for 24 hours each day, hourly during daylight, etc.); counts were made and expressed as beats per minute for each sampling interval.

Results for this test are normally expressed in terms of the concentration of test material that causes a statistically significant increase in the respiration rate for an individual fish. The number of fish (replication) necessary to determine this threshold concentration has not been clearly documented in the literature, but it may be assumed that within the practical limits of the researcher's resources maximum replication would provide greatest confidence.

The present study did not progress beyond studies of fish base-line responses to pure dilution waters, and no toxicants were tested.

RESULTS AND DISCUSSION

Initial trial runs were conducted to establish information on the variables which affect and characterize the background or base-line respiration rate. The experiments conducted to date were all done with dilution water in order to collect information on the following:

(a) Fish response variability

(b) Diurnal pattern and fluctuations

(c) Acclimation period

(d) Effect of static vs. flow conditions

(e) Effect of photoperiod

Four runs were made and four sets of data are available for discussion. These data are presented in detail in Appendix I. A typical recorded signal cannot be described, because considerable variation was noted. In Fig. 2, two examples of recorded signals are shown, with fish movement evident in one. The magnitude of the signal is a function of the distance of the fish from the the electrodes and is not useful as an indicator with this apparatus. The signal varies from an apparently simple looking spike to a peak with multiple components. This may be a function of recorder speed; a slower speed may reveal that the simple spike is actually a multiple peak response. For this work it was necessary only to be able to count distinct response signals.

VARIABILITY

The most apparent phenomenon observed during this series of base-line runs was that respiration rates vary widely between fish. In Table I a summary of respiration rate values by fish for each day of each run is presented. The mean respiration rates per day varied from one fish (Fish No. 1, Run 12/7) which

averaged a rate of 44 counts per minute over the experiment to another fish which averaged 150 counts per minute (Fish No. 6, Run 7/30). Both were in dilution water, both were healthy, and neither showed any signs of stress during the test.

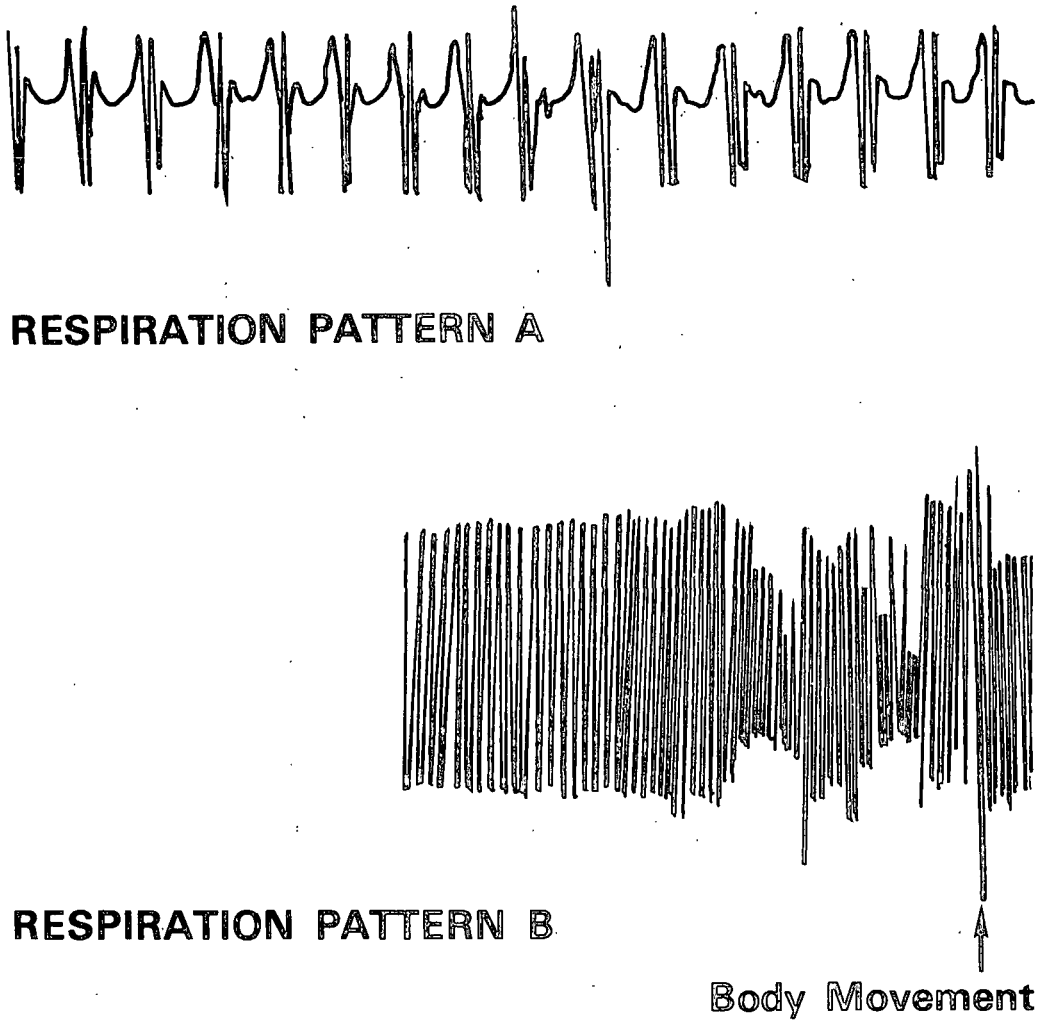

Figure 2. Examples of signals produced by bluegill monitoring apparatus.

TABLE I

A SUMMARY OF FISH RESPIRATION RATE DATA

Run No.	Fish No.	Mean Daily Respiration Rate						Standard Deviation					
		Day 1	Day 2	Day 3	Day 4	Day 5	Overall Mean	Day 1	Day 2	Day 3	Day 4	Day 5	Overall Mean
5/18	1	65	84	78	--	--	79	11	19	10	--	--	16
	2	131	82	72	--	--	89	29	9	8	--	--	26
	3	99	68	70	--	--	75	11	7	7	--	--	15
	4	90	85	89	--	--	87	11	19	17	--	--	15
	5	84	85	88	--	--	86	10	10	5	--	--	8
	6	104	90	113	--	--	99	1	9	23	--	--	16
7/30	1	58	42	44	38	70	49	13	7	15	12	26	17
	2	50	64	64	79	136	77	49	49	17	25	46	45
	3	66	56	58	61	56	59	10	6	2	14	9	8
	4	73	84	92	82	135	92	10	9	29	13	17	24
	5	63	51	47	48	73	55	19	14	4	18	48	22
	6	142	130	169	156	179	150	21	43	40	59	32	42
10/6	1	69	67	64	65	--	66	3	4	3	2	--	3
	2	65	73	75	67	--	70	2	11	7	7	--	8
	3	84	73	88	83	--	82	5	21	7	8	--	12
	4	57	95	81	94	--	84	7	19	2	7	--	17
	5	57	58	58	51	--	56	7	7	15	3	--	8
	6	57	62	61	87	--	68	3	7	5	26	--	17
12/7	1	40	39	45	50	45	44	7	5	9	7	2	6
	2	61	55	53	55	45	54	22	8	9	9	4	10
	3	56	64	62	61	51	59	8	5	16	8	5	8
	4	53	69	59	72	64	63	7	9	5	1	6	6

Analysis of variance results for fish comparisons - Experiment 1217

Comparison of daily counts for Fish No. 1 $F_{0.05[3,15]} = 2.67$ n. sig.

Comparison of daily counts for Fish No. 2 $F_{0.05[3,15]} = 1.39$ n. sig.

Comparison of daily counts for Fish No. 3 $F_{0.05[3,15]} = 1.60$ n. sig.

Comparison of daily counts for Fish No. 4 $F_{0.05[3,15]} = 3.72^{**}$ sig.

Comparison of all fish for Day 2 daylight data: $F_{0.05[3,20]} = 19.26^{**}$ sig.

For experiment 12/7, the fish were compared statistically using analysis of variance and found to be significantly different from one another (comparing daylight measurements on Day 2, $F_{0.05[3.2d]} = 19.26^{**}$). This was a consistent phenomenon throughout the four experiments.

Not only did the fish vary from each other, but there was also a difference in how much individual fish respiration rates varied over the course of a day. The standard deviation in Table I varied from 3 to 45.

Apparently, to obtain response data for this type of assay, individual fish response data will have to be tested for significant increases based on individual variability. In experiment 12/7 multiple counts were taken for each hourly sample period, and means and standard deviations were calculated for these data. This provided information which could be tested with greater confidence; however, it was enormously labor intensive. The advantages of automation are readily apparent with this system. The data for 12/7 were also used to test whether individual fish differed in mean respiration rates from one day to the next throughout the test. Differences in respiration rates for fish No. 1 between daylight periods for the first day experiment were not significantly different ($F_{0.05[3,15]} = 2.67$ n.s.). The same was true for fish 2 and 3. However, fish No. 4 did show a difference in rate for the 5 days (see Table I for F values). So, while the fish differed from each other with respect to respiration rate, they generally remained consistent throughout the exposure period.

DIURNAL VARIATION

Other studies have demonstrated that fish respiration rates vary, with a normal pattern throughout the day. The greatest differences occur between light and dark periods, since the fish become inactive during darkness and require a period of

time to return to full activity when light returns. In Fig. 3 and 4 the diurnal activity patterns of two fish from experiment 12/7 were plotted (with standard deviations included). These two fish represent the slowest and fastest average counts per minute for the experiment. The darkness period counts were noticeably lower than the light period counts. When mean light vs. mean dark period counts for all fish in experiment 12/7 are compared statistically using a t test, there was a significant difference between daylight and darkness counts ($t = 11.39$ significant at $P_{0.5}$).

It appears that attention to the time of day and light level when the data are collected will allow best comparisons when toxicants are evaluated.

AFFECT OF FISH SIZE ON RESPIRATION RATE

The only apparent difference between fish that might account for the different respiration rates was size. In Table II available fish weights were compared with mean respiration rates for daylight periods exclusive of a two count acclimation period. An application of linear regression analysis produced an $r^2 = 0.0012$, which showed a distinct lack of correlation. Within the sizes involved for bluegills, weight did not seem to affect respiration rates.

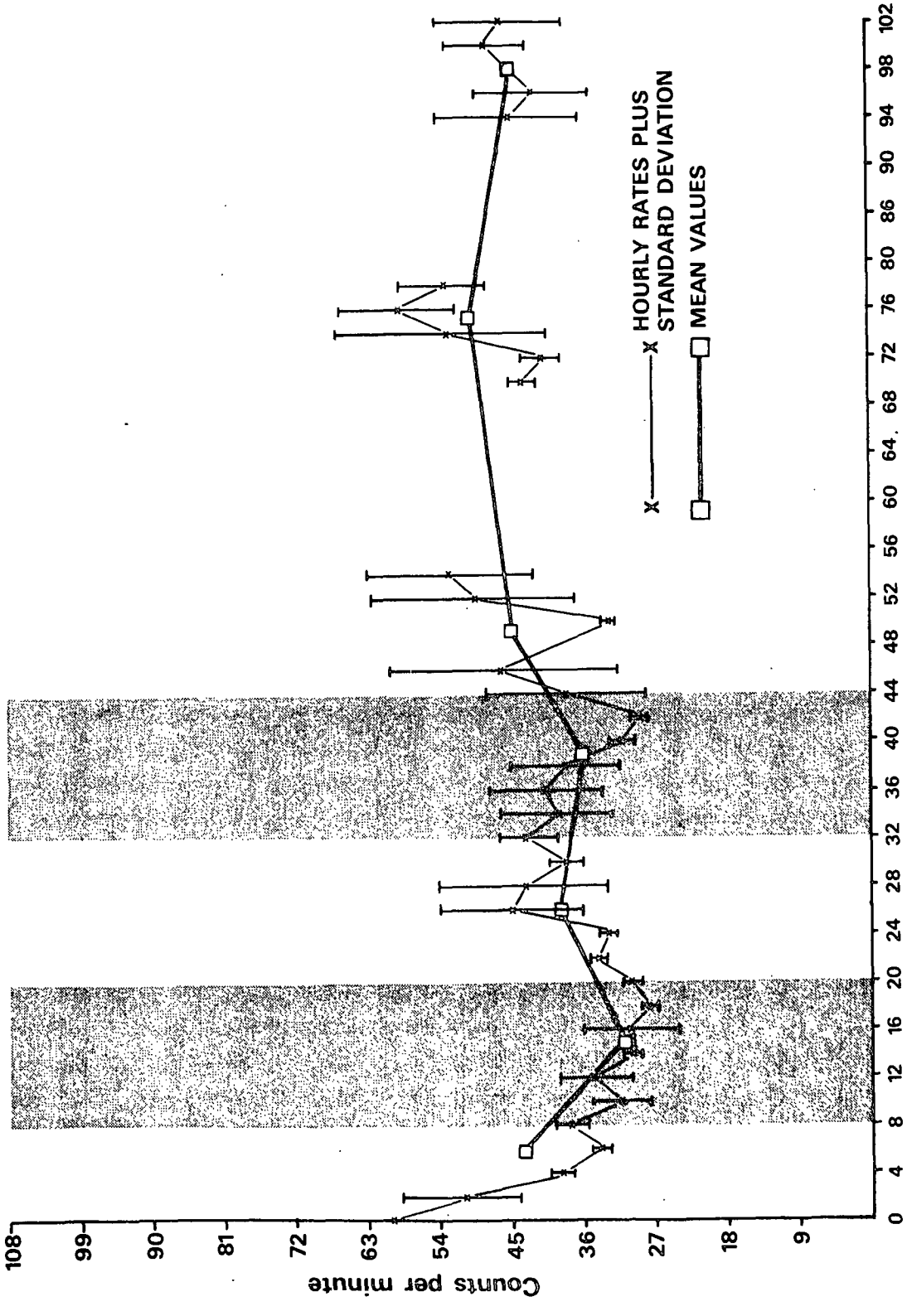

Figure 3. Mean respiration rates for fish No. 1 in experiment 12/7 showing diurnal variations.

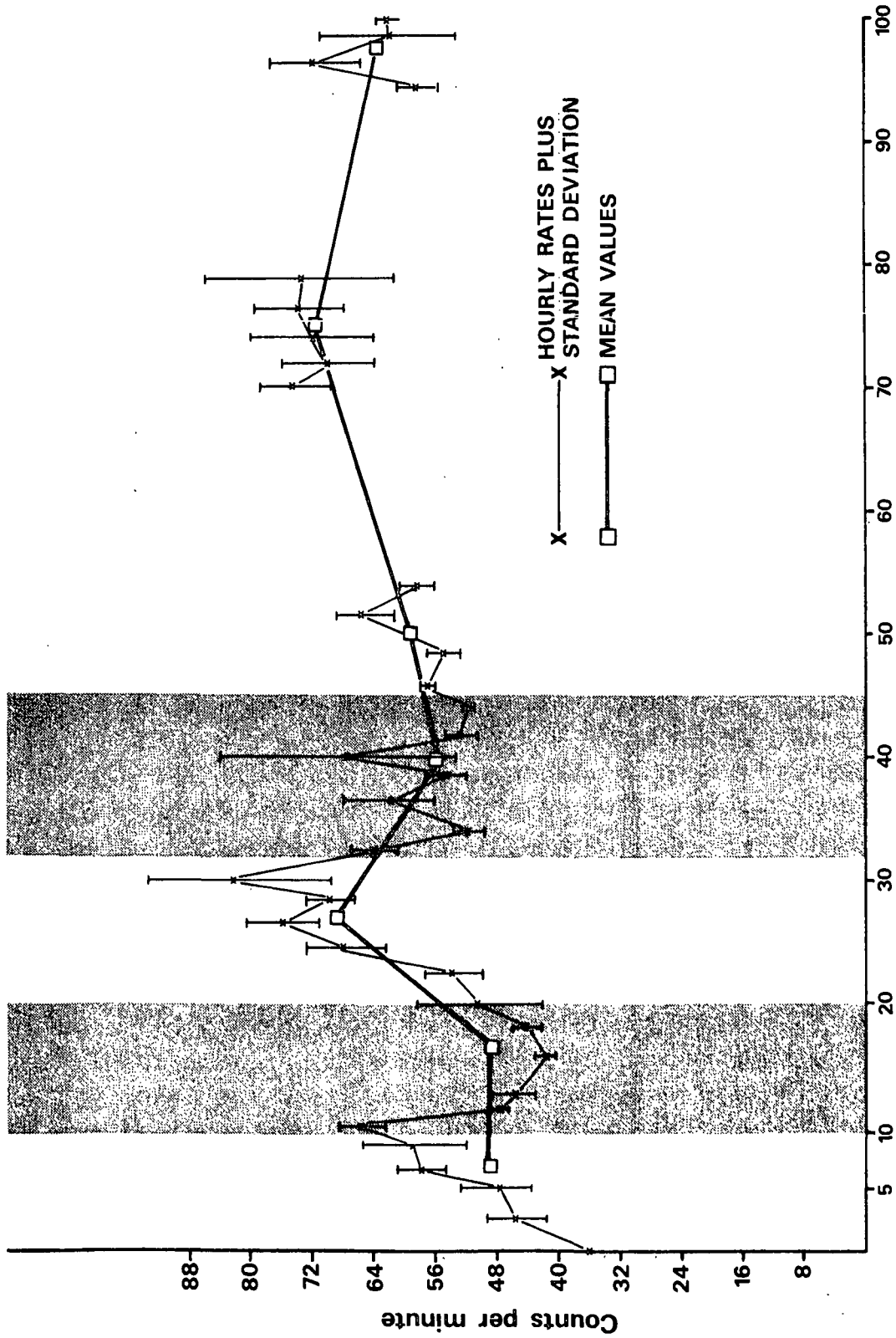

Figure 4. Mean respiration rates for fish No. 4 in experiment 12/7 showing diurnal variations.

TABLE II

EFFECT OF FISH SIZE ON RESPIRATION RATE

Assay	Fish No.	Fish Wt., mg	Mean Fish[a], rr counts/min	Static or Flow
5/18/81	1	300	78.5	F
	2	1100	89	F
	3	600	75	F
	4	600	87	F
	5	500	86	F
	6	600	100	F
July 30	1	2000	49	F
	2	1500	81	F
	3	900	64	F
	4	2000	93	S
	5	1000	50	S
	6	1000	152	S
Oct. 6	1	2200	68	F
	2	2100	69	F
	3	3200	81	F
	4	3000	82	F
	5	4400	60	F
	6	4300	65	F

$r^2 = 0.0012$, $n = 18$

[a]Values for daylight hours excluding counts during 4-hour acclimation period.

CONCLUSIONS

The preliminary base-line experiments conducted to evaluate this sublethal stress assay indicate that this method has promise as a tool for use with paper mill effluents. It is a labor-intensive or equipment-intensive test and will be expensive. However, it may provide rapid results, in contrast to a one- or two-year fish life cycle assay, which is also expensive. This is a tool for specialists and is not likely to be available for use in a mill setting by mill technical staff.

Considerable work remains to be done on the application of this particular approach to determine whether or not it will work with mill effluents and, if so, whether or not the resulting data are ecologically significant.

LITERATURE CITED

1. Randall D. T. and G. Shelton. 1963. The effects of change in environmental gas concentrations on the breathing and heart rate of a teleost fish. Comp. Biochem. Physiol. 9, 229-239.

2. Schaumburg, F. D., T. E. Howard, C. C. Walden. 1967. A method to evaluate the effects of water pollutants on fish respiration. Water Research (1):731-7.

3. Walden, C. C., T. E. Howard, and G. C. Froud. 1970. A quantitative assay of the minimum concentrations of kraft mill effluents which affect fish respiration. Water Research (4):61-8.

4. Davis, John C. 1973. Sublethal effects of bleached kraft pulp mill effluent on respiration and circulation in sockeye salmon (Oncorhynchus nerka). J. Fish. Res. Board Canada 30(3):369-77.

5. Cairns, J., Jr., K. L. Dickson, R. E. Sparks, N. J. Waller. 1970. A preliminary report on rapid biological respiration systems for water pollution control. J. Water Pollut. Control Fed. 42, 685-703.

6. Sparks, R. E., J. Cairns, Jr., A. G. Heath. 1972. The use of bluegill breathing rates to detect zinc. Water Research. Vol. 6, 895-911.

7. Spoor, W. A., T. W. Neiheisel, and R. A. Drummond. 1971. An electrode chamber for recording respiratory and other movements of free-swimming animals. Tran. Amer. Fish. Soc. 5(1):22-28.

8. Heath, Alan F. 1972. A critical comparison of methods for measuring fish respiratory movements. 1972. Water Research Vol. 6:1-7.

9. Goodman, D. A., and N. M. Weinberger. 1971. Submerged electrodes in an aquarium:validation of a technique for remote sensing of behavior. Behavior Research Methods & Instrumentation Vol. 3, 281-286.

10. Sloof, W. 1979. Detection limits of a biological monitoring system based on fish respiration. Bull. Environm. Contam. Toxicol. 23, 517-23.

11. Cairns, J. Jr., K. W. Thompson, and A. C. Hendricks. 1981. Effects of fluctuating sublethal application of heavy metal solutions upon the gill ventilatory response of bluegills (Lepomis macrochirus). EPA Project Report EPA-600/53-81-003. NTIS Order No. PB 81-150-997.

12. Cairns J., Jr. 1981. The use of mini computers in biology. The Biologist 63(2)33-47.

13. Gruber, D., J. Cairns, Jr., A. C., Hendricks. 1981. Computerized biological monitoring for demonstrating wastewater discharge. J. Water Poll. Control Fed. 53(4):505-11.

14. Cairns, J., Jr., D. Gruber. 1979. Coupling mini - and microcomputers to biological early warning systems. BioScience 29(11):665-668.

15. Gruber, D., J. Cairns, Jr., K. L. Dickson, A. C. Hendricks. 1929. A cinematographic investigation into the fish's bioelectric breathing Signal. J. Fish Biol. 14, 429-436.

16. Gruber, D., J. Cairns, Jr. 1981. Industrial effluent monitoring incorporating a recent automated fish biomonitoring system. Water, Air and Soil Pollution 15, 471-481.

17. Drummond, R. A., and W. F. Dawson. 1974. An inexpensive solid state amplifier for detecting movements and electrical potentials of fish. Transactions Amer. Fish. Soc. 103(2):391-392.

THE INSTITUTE OF PAPER CHEMISTRY

Eugene Zanella

Eugene F. Zanella
Group Leader
Bio-Research Group
Chemical Sciences Division

APPENDIX I

RESPIRATION RATE COUNTS FOR BASE-LINE EXPERIMENTS

TABLE III

EXPERIMENT 5/18

Time of Day	Elapsed Time, hr	Light (L) or Dark (D)	Fish No. 1	2	3	4	5	6
11:00								
13:00	0	L	73	152	107	98	91	105
15:00	1.5	L	57	111	92	83	77	103
18:00								
20:00								
22:00								
24:00								
02:00								
04:00								
06:00								
08:00	16.5	L	84.6	80	72	101	99	77
10:00	18	L	64.2	94	66	109	84	91
12:00	20	L	69	67	59	68	71	92
14:00	23	L	111	88	65	77	90	87
16:00	25	L	93	83	78	71	83	101
18:00								
20:00								
22:00								
24:00								
02:00								
04:00								
06:00								
08:00	42	L	66	70	71	70	94	132
10:00								
12:00	45	L	84	65	62	104	85	88
14:00	47	L	84	80	77	94	86	119
Fish wt., mg			300	1100	600	600	500	600

APPENDIX I (Continued)

RESPIRATION RATE COUNTS FOR BASE-LINE EXPERIMENTS

TABLE IV

EXPERIMENT 7/30

Time of Day	Elapsed Time, hr	Light (L) or Dark (D)	Fish No. 1	2	3	4	5	6
11:00	0	(24-hour	152	166	174	123	108	191
13:00	2	lights on)	73	101	77	--	85	145
15:00								
17:00	6	L	51	46	66	66	51	120
19:00	8							
21:00								
23:00	12	L	50	34	56	80	53	161
02:00								
04:00								
06:00								
08:00	20	L	39	29	61	76	50	95
10:00	22	L	56	33	52	72	48	103
12:00	24	L	35	25	48	84	43	111
14:00	26	L	40	26	62	86	42	103
16:00	28	L	40	152	55	89	43	181
18:00	30	L	48	97	63	82	82	203
20:00	32	L	40	88	54	98	49	113
22:00								
24:00								
02:00								
04:00								
06:00								
08:00	44	L	61	47	56	73	48	193
10:00								
12:00								
14:00	50	L	37	82	58	78	50	191
16:00	52	L	33	64	61	125	43	123
18:00								
20:00								
22:00								
24:00								
02:00								
04:00								
06:00								
08:00	70	L	46	77	52	97	69	190
10:00								
12:00	74	L	24	54	78	75	38	191
14:00								
16:00	78	L	45	105	54	73	36	88
18:00								
20:00								
22:00								
24:00								
02:00								
04:00								
12:00								
14:00	94	L	41	90	58	120	41	142
16:00	96	L	76	183	47	133	50	196
18:00	98	L	93	136	64	153	128	200

APPENDIX I (Continued)

RESPIRATION RATE COUNTS FOR BASE-LINE EXPERIMENTS

TABLE V

EXPERIMENT 10/6

Time of Day	Elapsed Time, hr	Light (L) or Dark (D)	Fish No. 1	2	3	4	5	6
11:00	0	L	106	70	84	71	118	57
13:00	2	L	67	64	89	52	62	60
15:00	4	L	71	67	79	62	52	55
17:00								
19:00								
21:00								
23:00								
02:00								
04:00								
06:00								
08:00	21	D	60	60	57	79	49	61
10:00	23	L	66	66	60	84	54	63
12:00	25	L	66	67	61	85	54	55
14:00								
16:00	29	L	73	85	97	117	66	68
18:00								
20:00								
22:00								
24:00								
02:00								
04:00								
06:00								
08:00	45	D	60	66	70	78	48	61
10:00	47	L	63	69	95	79	45	66
12:00								
14:00	51	L	61	89	89	84	74	56
16:00	53	L	67	80	80	81	54	62
18:00								
20:00								
22:00								
24:00								
04:00								
06:00								
08:00	67	D	58	64	80	71	72	53
10:00	69	L	63	59	81	86	48	71
12:00	71	L	66	72	92	95	53	73
14:00								
16:00	75	L	66	70	77	101	52	117
18:00								
20:00								
22:00								
24:00								
02:00								

APPENDIX I (Continued)

RESPIRATION RATE COUNTS FOR BASE-LINE EXPERIMENTS

TABLE VI

EXPERIMENT 12/7

Values Given are Means of 3 Readings (Standard Deviations) Taken During Each Period

Time of Day	Elapsed Time, hr	Light (L) or Dark (D)	Fish No. 1	2	3	4
09:00	0	L	61(30)	71(23)	52(15)	36(4)
11:00	2	L	50(8)	90(6)	46(8)	46(4)
13:00	4	L	39(1)	65(11)	52(6)	48(5)
15:00	6	L	34(1)	44(7)	63(1)	58(3)
17:00	8	L	38(2)	44(3)	63(2)	59(7)
19:00	10	D	31(3)	43(9)	45(4)	66(3)
21:00	12	D	35(4)	40(5)	48(3)	48(1)
23:00	14	D	30(1)	39(7)	45(8)	46(3)
02:00	17	D	30(6)	34(3)	39(0)	42(1)
04:00	19	D	28(1)	38(3)	41(3)	44(2)
06:00	21	D	30(1)	38(3)	43(2)	51(8)
08:00	23	L	34(1)	72(6)	67(12)	55(3)
10:00	25	L	33(1)	49(2)	53(11)	68(5)
12:00	27	L	45(9)	53(6)	66(8)	74(5)
14:00	29	L	42(10)	57(6)	65(3)	70(3)
16:00	31	L	38(2)	50(0.6)	68(12)	82(12)
18:00	33	L	43(4)	52(1)	65(13)	64(3)
20:00	35	D	39(7)	41(0.6)	51(3)	52(2)
22:00	37	D	40(7)	54(13)	62(7)	62(6)
24:00	39	D	38(7)	57(12)	52(4)	55(3)
02:00	41	D	31(1)	46(7)	53(5)	65(16)
04:00	43	D	29(1)	52(12)	45(2)	53(2)
06:00	45	D	37(10)	50(11)	59(20)	50(2)
08:00	47	L	46(14)	46(4)	53(2)	57(1)
10:00						
12:00	51	L	33(0)	48(2)	54(5)	55(3)
14:00	53	L	49(12)	52(2)	87(30)	66(6)
16:00	55	L	53(10)	67(22)	55(4)	59(2)
18:00						
20:00						
22:00						
24:00						
02:00						
04:00						
06:00						
08:00	71	L	44(2)	49(0.6)	55(11)	73(5)
10:00	73	L	41(3)	48(0.6)	63(20)	70(7)
12:00	75	L	52(14)	51(3)	54(4)	72(8)
14:00	77	L	59(7)	58(0.6)	60(7)	74(6)
16:00	79	L	52(5)	70(21)	73(21)	73(12)
18:00						
20:00						
22:00						
24:00						
02:00						
04:00						
06:00						
08:00	95	L	45(9)	43(3)	45(4)	58(3)
10:00	97	L	42(7)	47(3)	56(16)	72(6)
12:00	99	L				
14:00	101	L	48(5)	41(3)	54(10)	62(8)
16:00	103	L	46(8)	51(11)	51(6)	63(1)